Toxicity Testing Matrix: a working document for the Breast Cancer and Chemicals Policy (BCCP) Project

The “matrix” is a working document developed by the BCCP expert panel over the course of a day and half meeting. The Panel identified biological processes relevant to breast cancer starting at the cellular level and progressing up to the whole organism through generally recognized “hallmarks of cancer”. Existing toxicological test methods capable of measuring changes in each of these endpoints were identified and organized into the spreadsheet shown here. These included tests performed *in silico*, for example by computational Quantitative Structure Activity Relationship (QSAR), laboratory assays conducted *in vitro or in vivo*, and human epidemiological studies. Currently available validated assays were identified, as well as those that could be readily developed from methods currently used in breast cancer research by groups of researchers or individual labs. “Experimental” methods including investigations of high throughput toxicity tests were also identified.

The assays were assembled into a matrix—a chart that organized toxicity assays according to the endpoints they are designed to evaluate. This matrix served as a working document that the Panel used for the subsequent steps of the BCCP project, including development of a recommended approach to testing chemicals for their potential to alter breast cancer risk.

Because this document is the result of a brainstorm session and served as a basis for later steps in the project, it is not comprehensive and has not been peer-reviewed.

	Molecular Events Potentially Affecting Breast Cancer Development										
Model System for Evaluating Effects	**Alterations in Gene Expression**	**Cell cycle changes (proliferation; programmed cell death)**	**Genotoxicity**	**Development**	**Steroid hormones (Estrogen, Androgen, Progesterone)**	**Melatonin and circadian rhythms**	**Peptide Hormones (Growth hormones)**	**Metabolism Transporters**			
in silico	databases on gene expression microarrays (based on cell lines);	databases based on tissue arrays	models based on Ames test; other databases; Databases on CGH	databases on sNPs	QSAR; steroid receptor activity (agonist/antagonist); altered metabolism; transport protein interraction		database exists	database exists			
in vitro	RT-PCR (targeted gene expressions - ER, PR); microarray (pathway arrays); Western blots/proteomics. Examples of genes: EGF-receptor phosphorylation, p53 expression; estrogen metabolizing genes; E2 regulated genes; alternative RNA splicing; Aromatase promoter (breast specific) transcription binding assays (luciferase); Aromatase expression and activity assays	Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis;			Changes in activity of enzymes involved in steroid metabolism, and which should specify aromatase, 5-alpha reductase, and other p450 enzymes.		Caseins	Estrogen and adrenal metabolism genes (liver); Transporter assay kits			
bacterial			Ames test								
Primary cell culture/ extended explants	BrCa1 homozygous/heterozygous carrriers - could look for many endpoints in primary culture - e.g. p53 mutations, RT-PCR (targeted gene expressions - ER, PR); microarray (pathway arrays); Western blots/proteomics. Examples of genes: EGF-receptor phosphorylation, p53 expression; estrogen metabolizing genes; E2 regulated genes; alternative RNA splicing;Aromatase Promoter (breast specific) transcription binding assays (luciferase); Aromatase expression and activity assays	Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis;	microsatellite instabililty; LOH, GWAS; sNP, CGH; chromosomal aberrations, translocations, strand breaks, aneuploidy, Spectral karotyping (SKY); DNA adducts (agents or adducts of estrogen); MLA (autosomal assay for chromosomal changes - mouse lymphoma cells (general ca); HPRT (hypoxanthine (Shanaz); sister chromatid exchange; DNA repair		ELISA; Receptor binding assays; Receptor levels; Receptor isoforms; Receptor translocation (IHC, Flow); E-Screen, A-screen;		ELISA; Receptor binding assays; Receptor levels; Receptor isoforms; Receptor translocation (IHC, Flow);				
mammalian cell lines	RT-PCR (targeted gene expressions - ER, PR); microarray (pathway arrays); Western blots/proteomics. Examples of genes: EGF-receptor phosphorylation, p53 expression; estrogen metabolizing genes; E2 regulated genes; alternative RNA splicing; aromatase promoter MCF-10a luciferase; aromatase expression and activity assays; Estrogen/progesterone transcription assay with and without estradiol; microarray; w blots and proteomics; DNA Methylation patterns for epigenetic changes (methylation based arrays); MLA; methylation based sequencing ($$);	Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis;	microsatellite instabililty; LOH, GWAS; sNP, CGH; chromosomal aberrations, translocations, strand breaks, aneuploidy, Spectral karotyping (SKY); DNA adducts (agents or adducts of estrogen);		ELISA; Receptor binding assays; Receptor levels; Receptor isoforms; Receptor translocation (IHC, Flow); E-Screen, A-screen; transcriptional activation (in presence or absence of natural ligand)		ELISA; Receptor binding assays; Receptor levels; Receptor isoforms; Receptor translocation (IHC, Flow);				
organ cultures	DNA Methylation patterns for epigenetic changes (methylation based arrays); MLA; methylation based sequencing ($$);	Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis;	microsatellite instabililty; LOH, GWAS; sNP, CGH; chromosomal aberrations, translocations, strand breaks, aneuploidy, Spectral karotyping (SKY); DNA adducts (agents or adducts of estrogen);		Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, other perturbations)						
in vivo					metabolism, and which should specify aromatase, 5-alpha reductase, and other p450 enzymes.						
Whole animal (1- or 2-generation studies) also includes genetically modified breast cancer models	time points: pnd4 (culling); weaning; study endpoint 60 or 90d; carcinogen-induced steroidogenesis; mammary specific gene expression changes - correlate with body burden)	Ki67; cyclins, BrdU, (IHC, flow cytometry); apoptosis;	microsatellite instabililty; LOH, GWAS; sNP, CGH; chromosomal aberrations, translocations, strand breaks, aneuploidy, Spectral karotyping (SKY); DNA adducts (agents or adducts of estrogen);	Transgenic animal models - PPAR-KO; knockout models: IGF-1, PRL KO, ERKO, PRKO, p53, wnt KO	Uterotrophic assay (separate study design); leiomyoma models; uterine wt; whole mount of mammary gland, tissue changes and developmental staging (precocious or abnl development); altered branching patterns; circulating hormone levels; Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, other perturbations); pubertal protocol (rodent); zebrafish vitellogenin expression;	Ferrets for melatonin	Altered branching patterns; whole mount of mammary gland and developmental staging (precocious or abnl development); Dog models of mammary cancer	Estrogen and adrenal metabolism genes (liver); Transporter assay kits; analytic assays to assess levels of parent compound and metabolites in tissue; (in Naiive and carcinogen-induced model); strain specific transport and metabolism; N11KO models			
Human epidemiology longitudinal exposure data (NCS, Danish Natl birth cohort, BCERC, Ag Health study, Nurses Health study, EPIC, Million Women study, UK)	Buccal cell and buffy coat cells as DNA sources; Homogenous populations - New Zealand/Finland/Sweden (potential to look for breast ca); DNA methylation patterns for epigenetic changes		HLA based somatic mutation assays (flow cytometry) Exposure and DNA adducts; micronuclei; DNA repair		Potential biomarkers: hormonal levels, Steroid Receptor isoforms;	Occupational and other studies of breast ca rates with disrupted circadian rhythms. (e.g. Nurses Study)	Measure EGF-R, HER2/neu				

Model System for Evaluating Effects	Pathological markers	Phenotypic indicators TEB proliferation	Breast density	Ductal Hyperplasia	Atypical hyperplasia	Adenoma	DCIS	Carcinoma	Cell based changes				
in silico													
in vitro	nuclear/cytoplasmic ratios; IHC markers: calperin, cytokeratins, calponin, E-cadherin												
bacterial													
Primary cell culture/ extended explants	nuclear/cytoplasmic ratios; IHC markers: calperin, cytokeratins, calponin, E-cadherin												
mammalian cell lines	nuclear/cytoplasmic ratios; IHC markers: calperin, cytokeratins, calponin, E-cadherin	BrdU in malignant and high-risk cell lines											
organ culture													
in vivo													
Whole animal (1- or 2-generation studies; plus carcinogen-induced)	nuclear/cytoplasmic ratios; IHC markers: calperin, cytokeratins, calponin, E-cadherin	Whole mount study, or H&E stain, count TEB. Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, other perturbations)	Whole mount study, or H&E stain, count stromal v. epithelial %. Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, number of branch points, other perturbations)	Whole mount study, or H&E stain, Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, other perturbations)	Whole mount study, or H&E stain, Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, other perturbations)	Dog models; Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, other perturbations); lesions 1-2 mm are palpable in rodent	intraductal lesions - beaded epithelium (bridging) Epithelial/stromal changes (hyperplasia, hypertrophy, morphological changes, other perturbations); dog studies	Intralobular lesions - H&E stain; Dogs(susceptibilty differs by breed); Beluga whale data	Nipple/areola retention or abnl development; AGD				
longitudinal exposure data (NCS, Danish Natl birth cohort, BCERC, Ag Health study, Nurses Health study, EPIC, Million Women study. UK)	epithelial cells in breast milk as targets for testing.		Tanner staging; mammography; u/s	IHC; FNA; ductoscopy; ductal lavage	IHC; FNA; ductoscopy; ductal lavage	IHC; FNA; ductoscopy; ductal lavage	IHC; FNA; ductoscopy; ductal lavage. intraductal lesions - beaded epithelium (bridging)	Male cancer rates and body burden exposures; BRCA1 carriers and Ashkenazi Jewish population					

		Susceptibility factors							Biological Programs			Cancer Hallmarks					
Model System for Evaluating Effects	Obesity	Altered timing of breast development	Alterations in cyclicity	Breast perturbations	Metabolic factors	Metabolizing enzymes	age	Duration of estrogen exposure	Immune modulation	Oxidative stress	Evading apoptosis	Sustained Angio-genesis	Limitless replication potential	Tissue invasion/ metastasis	Insensitivity to anti-growth signals	Self-sufficiency in growth signals (autocrine)	
in silico					database exists	database exists			database exists	database exists				database exists			
in vitro										Protein adducts that fom as result of oxidative stress (eg. nitro-tyrosine); H2ax - dbl stranded breaks; 8-oxoguanine; lipid peroxidation assay - 4-HNE	TUNNEL; annexin V; cleaved caspase; apoptotic bodies; DNA ladders; (also could be used as HTPS)	activation of vEGF, FGF (co-culture); ECM - integrin (IPTG a-5 b-1); CD-49F (flow)	telomerase (flow); Morphological transformation, aneuploidy (non normal stem cell); tp53 suppression; cloning efficiency (not easy);	microarrays of /genes expressed in invasive breast ca; proteins Boyden (transwell) chambers - how cells move through the cells	TGF-b resistance	EGF indepedence; RNAi	
bacterial																	
Primary cell culture/extended explants																	
mammalian cell lines																	
co-culture									ROS levels	ROS levels					anti-differentiation (wagon wheel)	anchorage independent growth;	
3D organ culture									ROS levels	ROS levels		Factor 8 staining					
in vivo																	
Whole animal (1- or 2-generation studies))	Weight at birth, gain, etc. - live densitometry, abd fat	Whole mounts at PND4, weaning - H&E,	vaginal smears, age at 1st estrous reproductive capacity	onset and duration of lactation;Pup mortality due to impaired lactation	Wt, abdominal fat pads, Live densitometry, insulin resistance	alterations in expression			Whole mount lymphatic cells, evidence of lymphomas and thymomas; 8 -OH dG; WBC counts			activation of vEGF, FGF (co-culture)(may not be useful for breast ca); whole mount of vascularity; Factor 8 staining	Stem cell labeling assay (needs to be developed).	xenografts, whole body necropsy;			
Human epi		Thelarche, Tanner Staging + exposure history	Menarche + expousre history	Parity and gravida and duration of lactation		polymorphisms -		Epi studies of women on OCP + PM therapy	medical records for immune disease ;	mitochondrial diseases that are correlated with breast ca; milk samples for oxidative stress markers.				exposure + sample - look at families of genes associated with 'invasiveness'			